K-790

Fumarate Dismutation in *Desulfovibrio* G20 and the Effect of Formate

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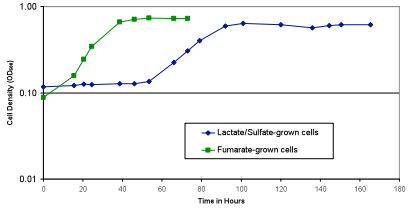
ENIGMA — Ecosystems and Networks Integrated with Genes and Molecular Assemblies

U.S. Department of Energy

Abstract

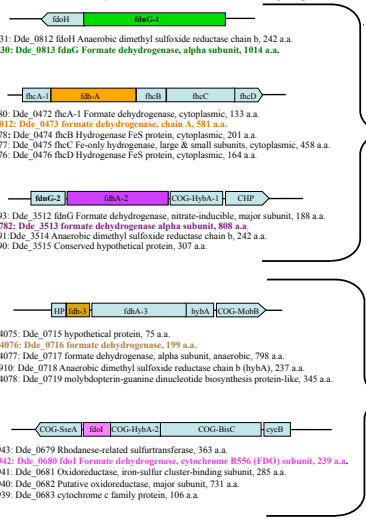
The anaerobic sulfate-reducing bacteria (SRB) of the genus *Desulfovibrio* are found in a remarkable variety of habitats, including soil, fresh water and salt water environments. SRB metabolism allows them to immobilize heavy toxic metals, such as uranium, through sulfide precipitation and/or through changing the redox state of the metal and thus its solubility. In studying the redox products of *Desulfovibrio* G20 during growth on various media, it was discovered a plasmid insertion mutant in the gene for the type-1 tetraheme cytochrome *c3*, *cycA*, was unable to grow on fumarate and the fumarate hydratase and fumarate reductase proteins were more than 10X decreased. Wildtype *Desulfovibrio* G20 anaerobic growth on fumarate appears to occur as a dismutation with the primary end products being succinate and acetate at approximately the theoretical ratio of 2:1, respectively. Yet wildtype growth with fumarate was inhibited with addition of as little as 5 mM formate. In wildtype G20, formate may inhibit growth directly by blocking an energy pathway or by down regulating the genes encoding the enzymes required for fumarate growth. Therefore the inability of the *cycA* mutant to grow on fumarate might result from an interruption in electron flow to the fumarate reductase or from an accumulation of inhibitory formate concentrations. To learn more about growth on fumarate, formate, or a combination of both, transposon mutants in formate dehydrogenases, formate C-acetyltransferases, malic enzymes, fumarate reductase, fumarate hydratase, and the pyruvate formate-lyase activating enzyme are being examined. Proteomic analysis of fumarate grown G20 cells, revealed that five of the eight proteins most increased in abundance compared to cells grown fermentatively on pyruvate, were specific to fumarate metabolism. Several of those highly expressed proteins are located in a single operon and include a fumarate reductase, fumarate hydratase and malic enzyme. To determine if the formate is having a regulatory effect on this operon, quantitative RT-PCR experiments are being performed to determine the expression of the genes in that operon.

Figure 1: Initiation of growth by *Desulfovibrio* G20 on Fumarate Requires Adaption to the Medium



When *Desulfovibrio* G20 is subcultured into Fumarate (60mM) medium with cells previously grown on Fumarate, the culture exhibited a short lag as compared to those which were innoculated with cells prviously grown in Lactate (60mM)/Sulfate (30mM) medium. It appears that an adaption by G20 must occur before lactate/sulfate cells will grow on fumarate medium.

Figure 2: *Desulfovibrio* G20 Operons of Annotated Formate Dehydrogenases



VIMSS395831: Dde_0812 fdoH Anaerobic dimethyl sulfoxide reductase chain b, 242 a.a.
VIMSS395830: Dde_0813 fdnG Formate dehydrogenase, alpha subunit, 1014 a.a.

VIMSS392980: Dde_0472 fhcA-1 Formate dehydrogenase, cytoplasmic, 133 a.a.
VIMSS3334012: Dde_0473 formate dehydrogenase, chain A, 581 a.a.
VIMSS392978: Dde_0474 fhcB Hydrogenase FeS protein, cytoplasmic, 201 a.a.
VIMSS392977: Dde_0475 fhcC Fe-only hydrogenase, large & small subunits, cytoplasmic, 458 a.a.
VIMSS392976: Dde_0476 fhcD Hydrogenase FeS protein, cytoplasmic, 164 a.a.

VIMSS393593: Dde_3512 fdnG Formate dehydrogenase, nitrate-inducible, major subunit, 188 a.a.
VIMSS3334782: Dde_3513 formate dehydrogenase alpha subunit, 808 a.a.
VIMSS393591:Dde_3514 Anaerobic dimethyl sulfoxide reductase chain b, 242 a.a.
VIMSS393590: Dde_3515 Conserved hypothetical protein, 307 a.a.

The operons of the three formate dehydrogenase transposons tested in this study (Figure 3A, 3B). FdnG-1 and FdnG-2 (Dde_0813 and Dde_3513, respectively) are located in the periplasm (Figure 6). Fdh-A (Dde_0473) is part of the cytoplasmic fhc complex. Operon and gene annotations are from http://www.microbesonline.org/.

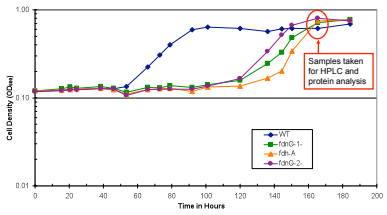
VIMSS3334075: Dde_0715 hypothetical protein, 75 a.a.
VIMSS3334076: Dde_0716 formate dehydrogenase, 199 a.a.
VIMSS3334077: Dde_0717 formate dehydrogenase, alpha subunit, anaerobic, 798 a.a.
VIMSS395910: Dde_0718 Anaerobic dimethyl sulfoxide reductase chain b (hybA), 237 a.a.
VIMSS3334078: Dde_0719 molybdopterin-guanine dinucleotide biosynthesis protein-like, 345 a.a.

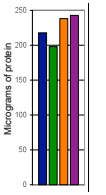
VIMSS395943: Dde_0679 Rhodanese-related sulfurtransferase, 363 a.a.
VIMSS395942: Dde_0680 fdoI Formate dehydrogenase, cytochrome B556 (FDO) subunit, 239 a.a.
VIMSS395941: Dde_0681 Oxidoreductase, iron-sulfur cluster-binding subunit, 285 a.a.
VIMSS395940: Dde_0682 Putative oxidoreductase, major subunit, 731 a.a.
VIMSS395939: Dde_0683 cytochrome c family protein, 106 a.a.

The operons of two additional annotated formate dehydrogenase in *Desulfovibrio* G20. The colored genes are transposons we have available for testing study (Figure 6). Fdh-2 (Dde_0716) is located in the periplasm, while fdoI (Dde_0473) is a membrane-bound protein of a formate dehydrogenase complex. Operon and genes annotations are from http://www.microbesonline.org/.

Figure 3: Growth on Fumarate Medium of *Desulfovibrio* G20 and Three Formate Dehydrogenases Transposon Mutants

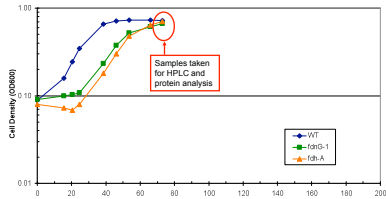
A. Subcultured from Lactate/Sulfate (60/30mM)

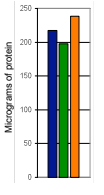




Substrate/ Metabolite	mM of Substrate/Metabolite at 165.5 hours			
	WT	fdnG-1-	fdh-A-	fdnG-2-
Fumarate	1.1	10.9	13.0	2.9
Succinate	27.2	20.5	20.4	23.1
Acetate	13.7	12.0	11.8	13.7
Malate	5.8	7.0	5.4	8.9
Pyruvate	0.15	0.3	0.4	0.3
Formate	0	0	0	0

B. Subcultured from Fumarate (60mM)



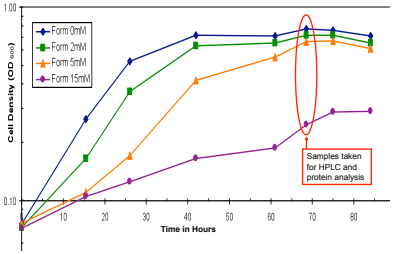


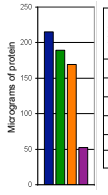
Substrate/ Metabolite	mM of Substrate/Metabolite at 73 hours		
	WT	fdnG-1-	fdh-A-
Fumarate	2.1	14.9	7.2
Succinate	25.6	19.5	22.8
Acetate	12.8	9.9	11.0
Malate	6.1	6.7	5.7
Pyruvate	0.5	0.2	1.5
Formate	0	0	0

Transposon mutants exhibited longer lag versus wildtype before initiating growth on fumarate, regardless of the source of the innocula. Ultimately, the wiltype and mutants exhibited final growth yields within 19% of each other. No unique trend in the end products was observed in the mutants compared to wildtype.

Figure 4: Inhibition of Growth of *Desulfovibrio* G20 on Fumarate by Formate

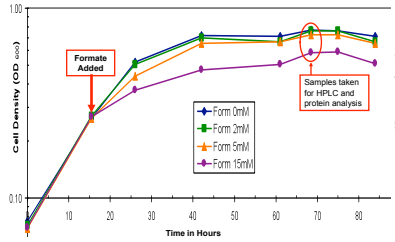
A. Formate (0mM, 2mM, 5mM, and 15mM) added at Time 0

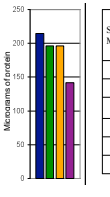




Substrate/ Metabolite	mM of Substrate/Metabolite at 73 hours			
	0mM Formate	2mM Formate	5mM Formate	15mM Formate
Fumarate	2.3	3.9	11.1	35.3
Succinate	25.3	24.8	23.1	12.7
Acetate	13.9	12.9	11.1	2.3
Malate	5.0	5.0	5.13	3.9
Pyruvate	0.2	0.2	0.2	0.2
Formate	0	0	0	0

B. Formate (0mM, 2mM, 5mM, and 15mM) added at 15.5 hours

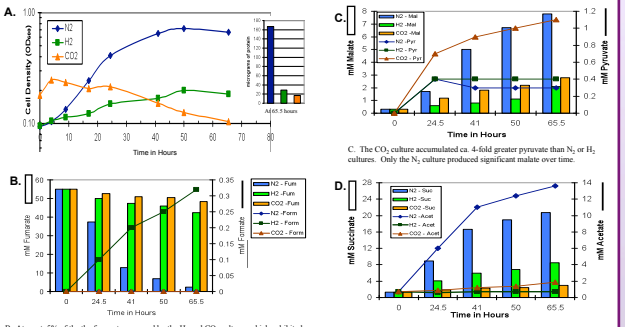




Substrate/ Metabolite	mM of Substrate/Metabolite at 73 hours			
	0mM Formate	2mM Formate	5mM Formate	15mM Formate
Fumarate	2.3	2.7	3.2	7.1
Succinate	25.3	25.5	25.6	25.2
Acetate	13.9	12.9	11.9	7.4
Malate	5.0	5.1	5.3	6.7
Pyruvate	0.2	0.2	0.2	0.2
Formate	0	0	0.1	0.1

Formate inhibited G20 growth on fumarate with the greatest effect observed when for was added at T_0' time. 15mM formate reduced final growth (µg/ml) by over 4-fold and proportionally produced less acetate: succinate then other treatments

Figure 5: *Desulfovibrio* G20 Growth on Fumarate (60mM) with N_2, H_2, or CO_2 in Headspace



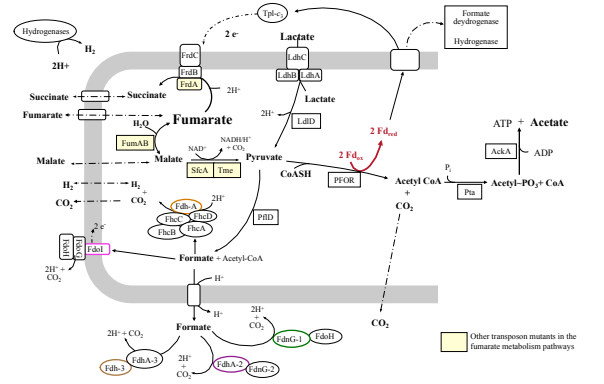
B. At most, 5% of the the fumarate was used by the H_2 and CO_2 cultures which exhibited only minimal growth yields (Fig. 5A). Interestingly, only the H_2 culture produced detectable amounts of formate.

C. The CO_2 culture accumulated ca. 4-fold greater pyruvate than N_2 or H_2 cultures. Only the N_2 culture produced significant malate over time.

D. Succinate and acetate accumulated at the theoretical 2:1 ratio in N_2 cultures. The H_2 culture began to accumulate succinate over time; however, no increase in acetate was observed

Desulfovibrio G20 Growth on Fumarate

Figure 6: Proposed Model for the Growth of *Desulfovibrio* G20 on Fumarate (60mM) and possible Inhibitions



Summary

- **G20 grows by dismutation of fumarate producing the theoretical ratio of 2:1 succinate:acetate endproducts.**
- **Formate dehydrogenase mutants grew more slowly than wildtypeG20 on fumarate with Fdh-A (Dde_0473) being more delayed.**
- **Growth of G20 on fumarate is inhibited by formate, H_2 and CO_2**
- **Formate was produced during growth of G20 on fumarate with H_2.**
- **The H_2 inhibition may be due to inability to reduce ferredoxin or a possible blockage of proton pumping allowing only the slight reduction of fumarate to succinate.**

Future Plans

- **Compare Fdh mutant growth to wildtype on fumarate with formate, H_2 and CO_2**
- **Perform qRT-PCR on genes in fumarate and formate dehydrogenases operons during different growth conditions**
- **Microarray analysis and proteomic analysis on fumarate-grown G20 cells are currently underway.**

Acknowledgements

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